

Amendments to the Specification

Please replace the paragraphs from page 4, line 14-page 4,  
line 23 with the following new paragraphs:

Thus in one embodiment of this aspect of the invention, there is disclosed a nucleic acid encoding the polypeptide of Fig 6 (SEQ ID NO:1).

*b* A genomic sequence corresponding to the *Arabidopsis* FRI locus is shown in Fig 4 (SEQ ID NO:2). A putative cDNA sequence transcribed from this genomic sequence is shown at Fig 5 (SEQ ID NO:3). Preferably the nucleic acid of the invention comprises the coding sequence within Fig 5 (SEQ ID NO:3) (bases 362-2188 inclusive). More preferably the nucleic acid comprises the sequence of Fig 5 (SEQ ID NO:3) or Fig 4 (SEQ ID NO:2).

Please replace the paragraph at page 5, lines 16-22 with the following new paragraph:

*b* Artificial variants (derivatives) may be prepared by those skilled in the art, for instance by site directed or random mutagenesis, or by direct synthesis. Preferably the variant nucleic acid is generated either directly or indirectly (e.g. via one or more amplification or replication steps) from an original nucleic acid having all or part of the sequence shown in Fig 5 (SEQ ID NO:3).

Please replace the paragraph at page 6, lines 30-35 with the following new paragraph:

*b* Homology may be over the full-length of the relevant sequence shown herein, or may be over a part of it, preferably over a

*B3*  
contiguous sequence of about or greater than about 20, 25, 30, 33, 40, 50, 67, 133, 167, 200, 233, 267, 300, 333, 400 or more amino acids or codons, compared with Fig 6 (SEQ ID NO:1) or 5 (SEQ ID NO:3) respectively.

Please replace the paragraph at page 6, line 37-page 7, line 9 with the following new paragraph:

*B4*  
Thus a variant polypeptide in accordance with the present invention may include within the sequence shown in Fig 6 (SEQ ID NO:1), a single amino acid or 2, 3, 4, 5, 6, 7, 8, or 9 changes, about 10, 15, 20, 30, 40 or 50 changes, or greater than about 50, 60, 70, 80 or 90 changes. In addition to one or more changes within the amino acid sequence shown, a variant polypeptide may include additional amino acids at the C-terminus and/or N-terminus. Naturally, changes to the nucleic acid which make no difference to the encoded polypeptide (i.e. 'degeneratively equivalent') are included.

Please replace the paragraph at page 7, lines 16-19 with the following new paragraph:

*B5*  
In a further aspect of the invention there is disclosed a method of producing a derivative nucleic acid comprising the step of modifying any of the sequences disclosed above, particularly the coding sequence of Fig 5 (SEQ ID NO:3).

Please replace the paragraph at page 9, lines 3-18 with the following new paragraph:

*B6*  
In another embodiment the nucleotide sequence information provided herein may be used to design probes and primers for probing or amplification of FRI or variants thereof. An oligonucleotide for use in probing or PCR may be about 30 or fewer nucleotides in length (e.g. 18, 21 or 24). Generally

specific primers are upwards of 14 nucleotides in length. For optimum specificity and cost effectiveness, primers of 16-24 nucleotides in length may be preferred. Those skilled in the art are well versed in the design of primers for use processes such as PCR. If required, probing can be done with entire restriction fragments of the gene disclosed herein which may be 100's or even 1000's of nucleotides in length. Naturally sequences may be based on Fig 4 (SEQ ID NO:2) or Fig 5 (SEQ ID NO:3), or the complement thereof. Small variations may be introduced into the sequence to produce 'consensus' or 'degenerate' primers if required.

Please replace the paragraph at page 25, lines 19-36 with the following new paragraph:

The promoter region or other control sequences may be readily identified on the basis of the genomic sequence shown in Fig 4 (SEQ ID NO:2) using a probe or primer as described above in relation to the isolation of variants. Generally they will be found 5' to the open reading frame of the gene and are obtainable by probing a genomic DNA library with a nucleic acid of the invention, selecting a clone which hybridizes under conditions of medium to high stringency, and sequencing the clone 5' to the open reading frame of the gene. Where only a small amount of sequence is present in the 5' region, this sequence may be used to reprobe the library to genome walk further upstream. Analysis of the upstream region will reveal control regions for gene expression including control regions common to many genes (i.e TATA and CAAT boxes) and other control regions, usually located from 1 to 10,000, such as 1 to 1000 or 50 to 500 nucleotides upstream of the start of transcription. Sequences identified as described above can be assessed for promoter activity.

Please replace the paragraphs at page 29, line 36 - page 30,

line 4 with the following new paragraphs:

Fig 4 shows the (late flowering) H51 genomic sequence of the first 17 kb of 84M13 encompassing the FRI gene (SEQ ID NO:2).

*AB*  
Fig 5 shows the likely cDNA sequence of the H51 FRI gene (SEQ ID NO:3).

Fig 6 shows the FRI amino acid sequence predicted from the cDNA sequence (SEQ ID NO:1).

Please replace the paragraph at page 34, line 23 - page 35,  
line 17 with the following new paragraph:

*AB*  
The H51 genomic sequence of the first 17 kb of 84M13 has been determined (FRI sequence in Fig.4, (SEQ ID NO:2)). RT-PCR experiments have been used to delimit the transcribed region using primers described in Table 2. Products were obtained using the primers at the 5' end CLFRI10 -UJ43 but not when CLFRI9 - UJ43 were used. This predicts the start of transcription to be between CLFRI-9 and CLFRI10 and there is a putative TATA box 20bp downstream of CLFRI9. The primers CLFRI11, CLFRI 1, CLFRI2, CLFRI3 all gave products with UJ43 that were the same size from cDNA made from polyA RNA isolated from H51 plants as from plasmid DNA indicating that there were no introns in this region. This then places the beginning of the open reading frame downstream of a stop codon at position 455 (Fig. 4, (SEQ ID NO:2)). The first MET codon is at position 574 (Fig 4, (SEQ ID NO:2)) giving a 5' untranslated leader of >360 nucleotides. By using RT-PCR we were able to verify that this gene is transcribed at least in leaves of both Li-5 and H51. Furthermore we were able to amplify the cDNA with primers UJH-UJ37 and to verify the positions of the first two introns by digestions of RT-PCR products (the second

splice site creating a *Dra*I restriction site). The next stop-codon in frame (2880, Fig. 4, (SEQ ID NO:2)) is likely to terminate translation. This stop-codon is well before the EcoRI site (15516) which defines the end of the complementing *FRI* gene in pJU226. RT-PCR analysis of the 3' end of the transcribed region yielded a product between UJ30 and an oligo dT primer of ~540bp putting the polyadenylation site around the primer CLFRI16. This would give a 3' untranslated region of ~500 nucleotides. The most likely transcribed sequence is shown in Fig. 5 (SEQ ID NO:3), the exact delimitation at both the 5' and 3' ends has still not been determined. The predicted amino acid sequence from this cDNA is shown in Fig. 6 (SEQ ID NO:1).

Please replace the table at page 40, line 18 - page 41, line 8 with the following new table:

Table 1

<u>MARKER,</u> <u>(SEQ ID NO)</u>	<u>CLOSE RFLP</u> <u>POSITION</u>	<u>BAC</u>	<u>PRIMER</u>	<u>SEQUENCE</u>	
UJ3-4	(4) (5)	CC27P11 98105-100090	F2N01	UJ3	GTC GGA CCA CAG TTG ATA AGA AT
			UJ4		TCG CAG ATA AGG AGA CTA ACC A
UJ8-9	(6) (7)	40D10 47043-472167	T18A10	UJ8	GAG TTC CGC GAC CCT TTA C
			UJ9		TAG TTT CCG TTG ATA TGT GAT TT
UJ10-11	(8) (9)	mi122 90704-91517	T18A10	UJ10	TAA GAA GCC GAA AAC AAA AGG AT
			UJ11		AGG GTA AAA ACT GCA GAT GAA AAT
UJ12-13	(10) (11)	mi51 105629-106219	F5I10	UJ12	CGG GGT CAG GTA ATA GCA CAC
			UJ13		GGT TTT CGG ATT TCG GAT TTT A
UJ14-15	(12) (13)	mi51-mi204 54494-55506	F5I10	UJ14	AAT TCA ACC GCA TCG TAT CAG
			UJ15		TAT CAG CCG TAT CAA CCA CAT T
UJ18-19	(14) (15)	mi51-mi204 49820-50835	F5I10	UJ18	CCA CCG TTA GTC TAT GCC TGA GTA
			UJ19		GAT GGG TCG GTG GGT GAA C

UJ20-2 (16) mi204-mi122 F6N23 UJ20 ACC GCA GAA GCA GCA TTA GC  
(17) 52046-53173 UJ21 CTC CGC GCA GGT GAT TTG  
 UJ24-25 (18) mi204-mi122 F6N23 UJ24 CTC CCG ACA GTT TCT TTG ACG  
(19) 45586-47168 UJ25 CCT GTT CCT GGC GGT GTA G

Please replace the table at page 42, line 14 - page 44, line 21 with the following new table:

Table 2

<u>OLIGO NAME</u>	<u>(SEQ ID NO:)</u>	<u>OLIGO SEQUENCE</u>	<u>POSITION IN GENOMIC SEQUENCE</u> (5' > 3')
UJF	<u>(20)</u>	AGT ACT CAC AAG TCA CAA C	1 > 19
CLFRI-8	<u>(21)</u>	GGG ATT ATC GTG TTT GAA G	49 > 67
CLFRI-9	<u>(22)</u>	CAT ATT ACC GAG CAA GAA C	130 > 149
UJO	<u>(23)</u>	CAG TGG TTT ATA ACA TGT C	183 > 165
CLFRI-10	<u>(24)</u>	CAT GTC GTA ATC ATG CAA C	213 > 231
CLFRI-11	<u>(25)</u>	GTG CGT AGA TTC AAT TAT TTG	276 > 296
CLFRI-1	<u>(26)</u>	CAA ATA CAT ATT TTC ATA AGC	349 > 370
UJG	<u>(27)</u>	CTA AAC ATA TAA CGA TTA CC	386 > 405
UJ41	<u>(28)</u>	CGT TTT CTC CTA ATT AAA AG	
UJ41-2	<u>(29)</u>	CGT TTT CTC CTA CTT AAA AG	420 > 439
CLFRI-2	<u>(30)</u>	CTT CAC AAT ATA CAG TTC A	477 > 495
CLFRI-3	<u>(31)</u>	GTG GAA ATT AGG GCT TCT G	529 > 547
RI-CLFRI-3	<u>(32)</u>	CCA GAATTC GTG GAA ATT AGG GCT TCT G	529 > 547
UJP	<u>(33)</u>	GTG GAT AAT TGG ACA TGA G	589 > 571
UJH	<u>(34)</u>	CCA TAG ACG AAT TAG CTG C	746 > 764

| B11  
Cents

UJ43	<u>(35)</u>	GAA GAT CAT CGA ATT GGC	801 > 794
UJ32	<u>(36)</u>	GGT TTA TTC GAC GTC TCC	1001 > 984
UJQ	<u>(37)</u>	GCT TTG AAA TTG GCC AAG G	1106 > 1123
UJ33	<u>(38)</u>	AGA CTC CAG TAT AAG AAG	1242 > 1225
UJ26	<u>(39)</u>	AGA TTT GCT GGA TTT GAT AAG G	1440 > 1461
UJ34	<u>(40)</u>	ATA TTT GAT GTG CTC TCC	1664 > 1647
UJ35	<u>(41)</u>	CTC AAA TGA CTC CTT GCT C	2058 > 2040
UJ28	<u>(42)</u>	TGC GAA AGA ACT ACC AGG ATG	2258 > 2278
CLFRI-6	<u>(43)</u>	CAG CTC TTG TGA GTA GTT AC	2527 > 2546
UJ29	<u>(44)</u>	ATT CAT ACT CTC CAG GTC A	2662 > 2680
UJ37R	<u>(45)</u>	AAC AAC AGT TAC CAT ATG G	2767 > 2785
UJ37	<u>(46)</u>	ACC ATA TGG TAA CTG TTG	2786 > 2769
UJ30	<u>(47)</u>	TTA TCC AAT CAA AGG TCT CC	2835 > 2854
CLFRI-13	<u>(48)</u>	GTC ATT TAT TTA ACT CCC AA	2932 > 2951
CLFRI-13R-RI	<u>(49)</u>	CGC GAATTC TTG GGA GTT AAA TAA ATG AC	2951 > 2932
CLFRI-14	<u>(50)</u>	GCT CCT GTA ATT GAC ATT TAA G	3003 > 3024
CLFRI-15	<u>(51)</u>	CAC TAT CTA AAT AGA CCT C	3077 > 3095
UJ44	<u>(52)</u>	TGC GGA TTC CAA CCT TG	3171 > 3187
CLFRI-12	<u>(53)</u>	GAT TGT CAA GCT CAA GTT GG	3298 > 3279
UJ38	<u>(54)</u>	CAA GAT CAA AGA CTG CTA AAT C	3360 > 3339
CLFRI-16	<u>(55)</u>	GTG AGT GTA TCT AGT GTT CA	3391 > 3372
UJ39	<u>(56)</u>	CAG AAG CCT CCG GCG AAC	3761 > 3744

Please replace the line at page 53, line 27 with the following new line:

b12

Landsberg erecta VRN2 cDNA (SEQ ID NO:57)

please replace the line at page 54, line 21 with the following new line:

Columbia VRN2 cDNA (SEQ ID NO:58)